The choice of the optimal technology of the controversial Saussurea extract, which stimulates the hematopoietic function of the bone marrow, having osteogenic and immunotropic activity

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The search of the best technology of Saussurea controversa extract, stimulating hematopoietic function of bone marrow possessing osteogenic and immunotropic activity

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SUMMARY

Extract of leaves of controversial saussurea (Saussurea controversa DC.) Exhibits osteogenic, immunomodulatory, anti-inflammatory activity and is a promising candidate for expanding the arsenal of means of complex conservative therapy of osteomyelitis. As a result of the study, the optimal parameters for the extraction of target components (flavonoids, chelidonic acid, minerals) of the controversial Saussurea extract were revealed. The optimal conditions for the extraction with 40% aqueous ethanol was a threefold extraction of leaves of a plant, crushed to 3–6 mm, in a ratio of 1:15 at 80 ° C for 60 min.

Keywords: Saussurea controversa DC, biological activity, extraction parameters.

RESUME

Extract of Saussurea controversa leaf possesses osteogenic, immunomodulatory and antiinflammatory activity and it is a promising candidate for complex conservative therapy of osteomyelitis. As a result of the study, the optimal parameters of extraction of target components (flavonoids, chelidonic acid, minerals) of the Saussurea controversa leaf were by determined. The optimal conditions for the extraction by 40% aqueous ethanol was a three-time extraction of crushed to 3–6 mm leaves of the plant in a ratio of 1:15 at 80 ° C for 60 min.

Keywords: Saussurea controversa DC, biological activity, extraction parameters.

INTRODUCTION

An urgent problem of modern medicine is the search for drugs for conservative therapy of osteomyelitis, preceding and following surgical treatment. As a means of complex treatment of osteomyelitis, there are promising drugs based on natural biologically active substances with osteogenic activity in combination with immunomodulatory, anti-inflammatory effects and low toxicity. So, polysaccharides showed high osteogenic activityGnaphalium affine

[1], flavonoids Epimedii Enhances [2], epigallocatechin gallate, and chlorogenic acid [3]. Plants of the genus Saussurea DC (family Asteraceae) are successfully used to treat diseases of the musculoskeletal system [4]. So, the viewSaussurea lappa is included in the Chinese Pharmacopoeia, onon its basis, a drug is obtained for the treatment of rheumatoid arthritis, which is standardized for chlorogenic acid and quercetin glycosides [5]. The genus Saussurea DC includes more than 350 different species native to Eurasia and Latin America [6]. On the territory of the Russian Federation, 115 species are found, mainly in the Urals, Siberia and the Far East [7].

One of the most popular species in Siberia is the controversial Saussurea (Saussurea controversa DC., Then - S. controversa) is a perennial herb that lives mainly in the forest, foreststeppe region, on meadow-steppe phytocenoses, rubble, stony slopes. Plant

has stems 25–100 cm in height, ragged-cobweb pubescent, often dark purple-brown. Leaves 2.5–9 cm in size on the upper side are green, white-tomentose below, uneven along the edge. Baskets are collected in a small number in a dense shield. The wrappers are tiled, cobweb-fluffy, their outer leaves are broadly ovate, shortly pointed, the inner ones are oblong, at the apex obtuse and densely woolly. All baskets at the top are blackish purple. Receptacle densely membranous. The flowers are purple-violet [6, 7] (Fig. 1).

Folk medicine uses leaf extracts S. controversa with glaucoma,pulmonary diseases, tuberculosis of the lungs and bones [8], as an analgesic, hemostatic, antirheumatic, wound healing agent and in gastrointestinal diseases [9-11]. S. controversa has long been used in folk medicine in Siberia, Buryatia, Mongolia for epilepsy, diarrhea, infectious diseases and diseases of the hepato-biliary system [12]. Experimental studies confirm hemostatic [13] and antimicrobial [14] activityS. controversa.

Extract S. controversa on 40% ethanol showed osteogenic, immunomodulatory and anti-inflammatory activity in osteomyelitis in vivo [15-17]. Use of the extractS. controversa during therapy with cefazolin promotes regression and localization of inflammatoryprocess in animals with experimental osteomyelitis, reduces the content of diene conjugates, the activity of alkaline phosphatase in the blood serum and TBA-active products in the muscle tissue of the affected area [15]. There is a normalization of the total number of leukocytes, monocytes and neutrophilic granulocytes in the blood of experimental animals [16].

With the course application of the extract S. controversa is markedly activatedregenerative processes in the bone tissue of rats with model osteomyelitis: the density of distribution of osteoblasts and the area of mature bone tissue increase in comparison with untreated animals, an improvement in the morphological picture of bone tissue is observed [17]. After applying the extractS. controversa in the bone marrow of rats with model osteomyelitis stimulation of granulopoiesis is observed, activation of the mitotic activity of granulocytes, an increase in the number of myeloblasts and mature forms of granulocytes, an increase in the total number of nucleated cells [17].

Described properties of the extract S. controversa are associated with its constituent flavonol glycosides, chelidonic acid and its complex compound with calcium [18, 19]. Composition of five quercetin glycosides isolated from leavesS. controversa, stimulated granulopoiesis and lymphopoiesis in the bone marrow and intensified reparative processes in the zone of experimental osteomyelitis [18]. Helidonic acid and its complex compound with calcium, called saukalchelin, significantly stimulatedin vitro growth of cell mass and differentiation of multipotent mesenchymal stromal cells (MMSC) into osteoblasts, followed by mineralization of a 21-day-old MMSC culture in a standard nutrient medium without osteogenic additives [19].

Rice. 1. Saussurea controversial (Saussurea controversa DC.) In natural habitat and herbarium specimen

Thus, the extract S. controversa is a promising candidate for expansionarsenal of means of complex conservative therapy of osteomyelitis, which has high activity. To create a new drug, it is necessary to develop an optimal production technology.

The aim of this work was to select the optimal parameters for extracting the target components of the controversial Saussurea extract.

MATERIALS AND METHODS

Leaves were used as the object of research. S. controversa, collected in July 2014 in the Irkutsk region. Air-dry plant material with a moisture content of 6.3 ± 0.1% was crushed and sieved through sieves with a hole diameter of 1–6 mm. The extraction of raw materials was carried out by repeated maceration with aqueous ethanol under heating, simulating the technological process in a laboratory reactor (Radley's, Great Britain) equipped with a heated water jacket and an RZR 2020 stirrer (Heidolph, Germany) at a speed of 300 rpm. The extracts obtained after each step of maceration were combined, the extractant was removed in a rotary evaporator at a temperature not higher than 50 ° C. The concentrated thick extract was dried by convective drying to a moisture content of no more than 5%. To select the optimal extraction parameters, we varied the ratio of raw material and extractant, the degree of grinding of the raw material, time, temperature and frequency of extraction. The yield of extractive substances (EV), ash content, and the content of flavonoids (FL) and chelidonic acid (CA) were evaluated.

To determine the content of flavonoids, 0.1 g (so-called) of the dry extract was dissolved in 40% ethanol in a 25 ml volumetric flask and brought to the mark with the same ethanol (solution A). 2 ml of solution A was placed in a 25 ml volumetric flask, 5 ml of 5% ethanol solution of aluminum chloride, 0.3 ml of concentrated hydrochloric acid were added, and the volume was adjusted to the mark with 95% ethanol. Optical density was measured on a spectrophotometer at a wavelength of 410 nm after 60 min. In parallel, the optical density of a solution of a standard sample of rutin was measured. To do this, 1 ml of a 0.02% rutin standard solution was placed in a 25 ml volumetric flask, 5 ml of a 5% ethanol solution of aluminum chloride, 0.3 ml of concentrated hydrochloric acid were added and brought to the mark with 95% ethanol.

Preparation of GSO rutin solution: 0.0050 g (accurately weighed) of GSO rutin, dried to constant weight at a temperature of 100–105 ° C, was dissolved in a 25 ml volumetric flask in a small amount of heated 95% ethanol and the volume was brought to the mark with the same

solvent.

The calculation of the content of the sum of flavonoids (%) was carried out according to the formula:

$$X\% = \frac{A \cdot M_0 \cdot 25 \cdot 25 \cdot 1 \cdot 100 \cdot 100}{A_0 \cdot M \cdot 2 \cdot 25 \cdot 25 \cdot (100 - w)} = \frac{A \cdot M_0 \cdot 5000}{A_0 \cdot M \cdot (100 - w)},$$

where A is the optical density of the test solution; AO - optical density of GSO routine (average of three measurements); MO - mass of GSO routine, g; M is the mass of the sample of the extract, g; W is the moisture content in the extract,%.

To determine the content of chelidonic acid, 0.25 g (so-called) of the dry extract was placed in a conical flask and dissolved in 15 ml of water under weak heating. After cooling, two drops of phenolphthalein were added and titrated with 0.1 M sodium hydroxide solution until raspberry brown. 1 ml of 0.1 M sodium hydroxide solution corresponds to 9.205 mg of chelidonic acid.

RESULTS AND DISCUSSION

To select the optimal extractant of the target substances, we used water and aqueous ethanol of various concentrations of 20, 40, and 70% under the same extraction conditions. The yield of flavonoids increased with an increase in the extraction of ethanol, while the content of mineral components and extractives was significantly lower during the extraction with 70% ethanol (Table 1). The maximum yield of chelidonic acid was obtained by extraction with 40% aqueous ethanol. In addition, the same extract previously showed activity in biological experiments [15–17]. Thus, 40% aqueous ethanol is the optimal extractant of the target components from the leaves.S. controversa and was chosen as an extractant for further research.

Analysis of the results of extraction with 40% aqueous ethanol showed that the maximum yield of ER was observed in experiments 2, 5, and 11 (Table 2). A decrease in the frequency of extraction, the ratio of raw material and extractant with a simultaneous increase or decrease in the extraction time led to a decrease in the yield of EV. A decrease in the extraction temperature to 60–70 ° C or its increase to 90 ° C had the same effect. The maximum yield of EV was observed during the extraction of the fraction of the raw material passing through holes with a diameter of 1 mm, screened out from dust (experiment 11). This phenomenon can be explained by the peculiarity of the leaf structure of the controversial Saussurea: the lower epidermis of the leaf is abundantly covered with filamentous trichomes forming a felt covering [20]. Therefore, even when grinding the raw material, its particles stick together by means of hairs, forming larger ones. The bulk of the raw material (about 98%) is sieved only through sieves with holes 3–6 mm in diameter. Particles of raw material free from hairs pass through holes with a diameter of 1 mm, as a result of which they contain a greater amount of EV.

Table 1

The influence of the extractant on the content of target substances in the extract of Saussurea controversial (n = 3, p = 0.95)

Extractant	Extractive substances, %	Ash content,%	Flavonoids, %	Helidonic acid,%
Water	42.00 ± 0.40	27.2 ± 0.82	1.67 ± 0.05	10.18 ± 0.38
20% ethanol	39.20 ± 0.32	27.8 ± 0.84	1.44 ± 0.05	8.53 ± 0.09
40% ethanol	39.40 ± 0.30	26.15 ± 0.16	1.83 ± 0.03	13.20 ± 0.09
70% ethanol	31.00 ± 0.20	20.0 ± 0.60	1.89 ± 0.04	11.87 ± 0.09

table 2

Effect of extraction parameters with 40% aqueous ethanol on the content of target substances in the extract of the controversial Saussurea (n = 3, p = 0.95)

No.	Corresponding change	Krat- ness	Time, min	T, °C	The size particles, mm	EV,%	Ash content, %	FL,%	HC,%
1	1:15 1:10 1:10	3	thirty	80	3-6	39.40 ± 0.20	23.89 ± 0.15	1.50 ± 0.40	13.10 ± 0.05
2	1:15 1:10 1:10	3	60	80	3-6	45.10 ± 0.30	25.11 ± 0.11	1.73 ± 0.27	12.91 ± 0.20
3	1:15 1:10 1:10	3	90	80	3-6	43.40 ± 0.30	23.13 ± 0.10	1.64 ± 0.33	12.75 ± 0.04
4	1:15 1:10	2	60	80	3-6	39.20 ± 0.22	23.45 ± 0.11	1.53 ± 0.35	12.82 ± 0.05
5	1:15 1:15 1:15	3	60	80	3-6	45.70 ± 0.31	24.18 ± 0.12	1.79 ± 0.20	13.20 ± 0.09
6	1:20 1:15 1:15	3	60	80	3-6	43.95 ± 0.29	24.58 ± 0.10	1.48 ± 0.20	11.14 ± 0.11
7	1:10 1: 8 1: 8	3	60	80	3-6	34.40 ± 0.15	23.82 ± 0.15	1.64 ± 0.15	12.66 ± 0.03
eight	1:15 1:10 1:10	3	60	90	3-6	43.60 ± 0.25	23.62 ± 0.12	1.54 ± 0.25	12.53 ± 0.03
nine	1:15 1:10 1:10	3	60	70	3-6	41.42 ± 0.25	23.42 ± 0.10	1.69 ± 0.36	12.85 ± 0.05
ten	1:15 1:10 1:10	3	60	60	3-6	38.86 ± 0.18	24.95 ± 0.11	1.54 ± 0.09	12.84 ± 0.15
eleven	1:15 1:10 1:10	3	60	80	1	46.30 ± 0.25	21.95 ± 0.10	1.30 ± 0.12	12.94 ± 0.02
12	1:15 1:10 1:10	3	60	80	2-3	38.80 ± 0.15	26.15 ± 0.16	1.37 ± 0.18	13.06 ± 0.06
13	1:15 1:10 1:10 1:10	4	60	80	3-6	40.06 ± 0.15	23.59 ± 0.12	1.71 ± 0.30	12.92 ± 0.23

The ash content of the extract indirectly indicates the content of calcium, since it predominates in the mineral composition of the plant [21]. In general, all extraction options allow for the extraction of approximately equal amounts of mineral components. But threefold extraction for 60 min at 80 °C at a certain ratio of raw materials and extractant allows to slightly increase their yield (experiments 2 and 12). Similar conditions made it possible to increase the yield of chelidonic acid (experiment 5). The yield of flavonoids under similar conditions increases with an increase in the ratio of raw materials and extractants (experiments 2, 5, 13).

Thus, the optimal conditions for increasing the yield of target substances at

extraction with 40% aqueous ethanol from leaves S. controversa is a triple extraction,crushed to 3–6 mm, in a ratio of 1:15 at 80 ° C for 60 min.

CONCLUSIONS

1. Ethanol extract of leaves of controversial Saussurea (Saussurea controversa DC.) Is a promising candidate for expanding the arsenal of complex conservative therapy for osteomyelitis.

2. Optimal conditions for extraction with 40% aqueous ethanol (n = 3, 1:15, t = 60, 80 ° C) allow to increase the yield of extractives, incl. target components - flavonoids, chelidonic acid and minerals.

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